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Impaired irinotecan biotransformation in hepatic microsomal fractions from patients with chronic liver disease

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The anticancer agent irinotecan is a prodrug that is hydrolyzed by hepatic carboxylesterase to its active and toxic metabolite SN-38 and oxidized by CYP3A4 to its inactive metabolite APC.
- Irinotecan therapy is complicated by co-administered drugs that inhibit CYP3A4 and decrease APC formation and that indirectly increase SN-38 formation.
- Dose adjustment in cancer patients with liver disease has been recommended.

WHAT THIS STUDY ADDS

- In microsomal fractions from patients with severe hepatic dysfunction both APC and SN-38 formation were decreased due to down-regulation of CYP3A4 and carboxylesterase enzymes. Thus relative SN-38 : APC formation was preserved.
- In some fractions the SN-38 : APC ratio was increased, thus providing a possible explanation for clinical reports of increased SN-38 exposure in some patients with liver dysfunction.
- Close monitoring of SN-38 formation in patients with severe liver disease is warranted.

AIMS

Dose modification with the anticancer agent irinotecan is recommended in patients with severe liver dysfunction. This study evaluated the impact of liver disease on the relative formation of phase I products of irinotecan biotransformation in human microsomes *in vitro*.

METHODS

Microsomes from subjects with normal liver function and liver dysfunction ($n = 20$) were assessed for irinotecan biotransformation and the expression of cytochrome P450 (CYP) 3A4 and carboxylesterase (CES) enzymes.

RESULTS

Liver disease down-regulated CYP3A4 expression (median 33% of control, range 0–126%, $P < 0.05$) and impaired CYP3A4-dependent oxidation of irinotecan to the inactive 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) (median 0.2, range 0–1.21 pmol mg protein^{-1} min^{-1} compared with median 0.66, range 0–2.35 in control, $P < 0.01$). CES-mediated hydrolysis of irinotecan to 7-ethyl-10-hydroxycamptothecin (SN-38) was also impaired in liver disease (median 8.38, range 0–20.7 pmol mg protein^{-1} min^{-1} compared with median 13.3, range 0–28.9 in control, $P < 0.05$). In seven of 20 liver disease microsomes neither metabolite was detected but in three the SN-38 : APC ratio was high (41–68) compared with the remaining 10 samples (ratio 11–36).

CONCLUSIONS

Down-regulation of CYP3A4 in liver disease decreased APC formation from irinotecan. SN-38 production was decreased and CES1 and 2 were down-regulated in most samples. However, in a subset of disease samples SN-38 production was relatively high because CYP3A4 activity was markedly impaired. This may account for clinical reports of increased SN-38 exposure in some patients with liver disease. Dose adjustments in cancer patients with liver disease who receive irinotecan are important and circulating SN-38 concentrations should be monitored closely.

Introduction

The camptothecin derivative irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin) is important in the treatment of colorectal cancer. Irinotecan biotransformation is complex and involves activation to 7-ethyl-10-hydroxycamptothecin (SN-38) by human hepatic carboxylesterase (CES) enzymes and cytochrome P450 (CYP) 3A4-dependent oxidation to the inactive metabolite 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) [1–3]. SN-38 mediates the therapeutic actions of irinotecan as well as its principal dose-limiting toxicities of diarrhoea and neutropenia [3, 4]. The terminal UGT1A1-dependent glucuronidation of SN-38 occurs prior to excretion in bile by transporter proteins [5].

In man, the biotransformation of irinotecan is relatively inefficient and highly variable [3]; wide inter-individual variation in pharmacokinetic behaviour is also observed [6]. It has been reported that CYP3A4 inhibition *in vivo* decreases APC formation and increases the systemic response to SN-38 [7]. Similarly, clinical studies have reported that the dose-normalized exposure to SN-38 and its associated toxicity are increased in patients with liver disease [8–13]. Thus, extreme caution with irinotecan has been recommended in patients with liver disease and involves dose adjustment to minimize toxicity and maximize efficacy [11, 13]. However, the impact of liver disease on CES- and CYP3A4-mediated biotransformation of irinotecan has not yet been established directly. The present study assessed irinotecan biotransformation in microsomal fractions from patients with normal and impaired liver function. The principal finding to emerge was that CES1/2 and CYP3A4 were down-regulated in liver disease and impaired the relative formation of the major phase I metabolites SN-38 and APC.

Methods

Drugs and chemicals

Irinotecan injection concentrate (25 mg ml^{-1}) was a gift from Mayne Pharma (Mulgrave, VIC, Australia) and SN-38 and APC were generously provided by Pfizer (West Ryde, NSW, Australia). Camptothecin, CYP inhibitors and biochemicals were from Sigma Aldrich (Castle Hill, NSW, Australia) or Roche Pty Ltd (Castle Hill, NSW, Australia). Microsomal fractions containing cDNA-directed CYPs expressed in human B-lymphoblastoid or insect cells (Supersomes) were obtained from BD Biosciences (North Ryde, NSW, Australia). Reagents for electrophoresis were from Bio-Rad (Richmond, CA). HPLC-grade solvents were from Rhone-Poulenc (Baulkham Hills, NSW, Australia) and analytical reagents were from Ajax (Sydney, NSW, Australia). Hyperfilm-MP, Hybond-N$^+$ filters, and reagents for enhanced chemiluminescence were from Amersham GE Healthcare (Rydalmere, NSW, Australia). The preparation and characteristics of the anti-CYP3A4 and anti-CYP2D6 peptide antibodies have been reported elsewhere [14]. Anti-CES1 and CES2 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Liver donors and preparation of microsomal fractions

This study was approved by institutional ethics committees. 'Control' human liver was obtained from adult transplantation donors or as biopsies from the normal margin during liver resection (Queensland and Australian Liver Transplant Programs, Princess Alexandra Hospital, Brisbane, Queensland, and Royal Prince Alfred Hospital, Sydney, NSW, Australia, respectively). Cirrhotic tissue was obtained from individuals who had been diagnosed with hepatocellular or cholestatic liver disease. The severity of liver disease was graded according to the Child-Pugh system [15, 16]. Tissue was perfused with cold Viaspan solution (DuPont, Wilmington, DE, USA) and transported on ice to the laboratory. Liver microsomes were prepared as described [17] and protein was determined according to Lowry *et al*. [18].

Drug biotransformation in human liver microsomes

Incubations (0.5 ml) contained irinotecan (5 μM), microsomal protein (100 μg) and NADPH (1 mM) in potassium phosphate buffer (0.1 M, pH 7.4). Reactions were run at 37°C for 20 min and were terminated with cold acetonitrile : methanol (1 : 1, 1 ml). Irinotecan, SN-38 and APC were quantified by the LC-MS/MS assay of d'Esposito *et al*. on an Alltima C_{18} 5 μ column (150 × 2.1 mm; Alltech Associates, Castle Hill, NSW, Australia) coupled to an Agilent 1090 system [19]. Irinotecan biotransformation was linear under these conditions.

Microsomal midazolam 1'-hydroxylation and dextromethorphan *O*-demethylation activities in human hepatic fractions were measured as described elsewhere [20, 21].

Immunoquantification of microsomal proteins

Hepatic microsomes (10 μg for CYP3A4, 20 μg for CES1/2 and 25 μg protein for CYP2D6) were resolved on 10% sodium dodecyl sulphate-polyacrylamide gels (2 h at 120 V), transferred electrophoretically to Protran nitrocellulose (Schleicher and Schuell, Dassel, Germany) and then subjected to Western immunoblot analysis as described previously [17]. Immunoreactive proteins were detected by enhanced chemiluminescence (GE Healthcare, Chalfont St. Giles, UK) on Hyperfilm-ECL (Amersham, Little Chalfont, UK) and the resultant signals were analyzed by densitometry (Bio-Rad, Richmond, CA). Signal intensity was linear under these conditions.

Table 1
Control patients: drug histories and microsomal enzyme activities

	Drug history	Microsomal activity (pmol mg protein^{-1} min^{-1}) SN-38 formation	APC formation	Midazolam 1′-hydroxylation	Dextromethorphan *O*-demethylation
CL1	Dopamine, desmopressin	25.8	1.78	1.07	36.0
CL2	Flucloxacillin, ceftriaxone	11.2	1.05	4.31	46.9
CL3	Ranitidine, ampicillin, epinephrine	9.1	1.88	0.91	1.3
CL4	Dopamine, desmopressin, dexamethasone	13.4	0.56	0.79	11.0
CL5	Unknown	16.8	0.43	0.06	6.6
CL6	Dopamine	17.1	0.50	0.37	0
CL7	Sucralfate	28.9	1.02	1.03	21.2
CL8	Unknown	20.2	0.60	0.67	68.4
CL9	Simvastatin	23.8	2.06	1.58	2.9
CL10	Cefotaxime	19.4	2.35	1.73	230.7
CL11	None	17.4	0.56	0.45	8.8
CL12	Unknown	7.5	0.81	1.03	13.8
CL13	None	16.3	0.71	0.30	33.2
CL14	Unknown	0	0	1.30	7.6
CL15	Unknown	13.3	0.98	1.81	149.2
CL16	Unknown	11.2	0.78	0.34	59.7
CL17	Unknown	8.3	0.26	1.08	47.0
CL18	Unknown	0	0	0.62	46.2
CL19	Unknown	11.0	0.28	0.50	21.2
CL20	Unknown	0	0	0.23	219.6
Median		13.3	0.66	0.85	27.5
Range		(0–28.9)	(0–2.35)	(0.06–4.31)	(0–230.7)

CL, control liver (number), SN-38, APC.

Statistical analyses

Shapiro-Wilk testing established that the data were not normally distributed. Differences between samples from control and liver disease patients (20 per group) were detected with the Mann–Whitney *U*-test. Spearman's correlation coefficient was used to evaluate nonparametric regressions. Statistical analysis was performed with Statview (Abacus concepts, Berkeley, CA).

Results

Biotransformation of irinotecan in microsomal fractions from patients with normal hepatic function

Hydrolysis of irinotecan to SN-38 follows biphasic kinetics while oxidation to APC is monophasic. The K_m values for the low-affinity and high-affinity pathways of irinotecan hydrolysis were reported to be ~150 and ~2 μM, respectively [2, 22], while the K_m for APC formation in human hepatic microsomes was 40 μM [1]. However, although information on the unbound intrahepatic concentration in patients during therapy would be most informative, it is impractical to measure this. Instead, clinically relevant concentrations of irinotecan in patient serum are around ~2–4 μM [13, 23]. Thus, in the present study irinotecan biotransformation was estimated at a concentration of 5 μM.

The formation of SN-38 varied in hepatic microsomes from control subjects between 0.0 and 28.9 pmol mg protein^{-1} min^{-1} (median: 13.3 pmol mg protein^{-1} min^{-1}; $n = 20$, Table 1). The expression of both CES1 and CES2 immunoreactive protein was decreased in liver disease (Figure 1A). APC formation ranged from 0.0 to 2.35 pmol mg protein^{-1} min^{-1} (median 0.66 pmol mg protein^{-1} min^{-1}). Table 1 also presents midazolam 1′-hydroxylation and dextromethorphan *O*-demethylation activities measured in microsomes from individuals with normal hepatic function ($n = 20$). APC formation was correlated with CYP3A4-dependent midazolam 1′-hydroxylation ($\rho = 0.518$, $P < 0.05$) but not with CYP2D6-mediated dextromethorphan *O*-demethylation. Irinotecan was oxidized to APC by cDNA-expressed CYP3A4 (0.15 ± 0.01 pmol pmol CYP^{-1} h^{-1}) but not by CYPs 1A1, 1A2, 1B1, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A5, 3A7 or 4A11 (not shown). SN-38 was not generated from irinotecan by any CYPs. We also tested the impact of CYP inhibitors on the relative formation of SN-38 and APC (Figure 2A). The CYP3A-specific inhibitor troleandomycin decreased microsomal APC formation to 45 ± 6% and 13 ± 1% of control at concentrations of 100 μM and 250 μM, respectively, but SN-38 formation was unchanged from control. However, at the highest concentration of troleandomycin (500 μM), which essentially abolished APC formation (~5% of control activity), a small but significant increase in SN-38 formation was noted (by 24 ± 8% over control, $P < 0.01$). In

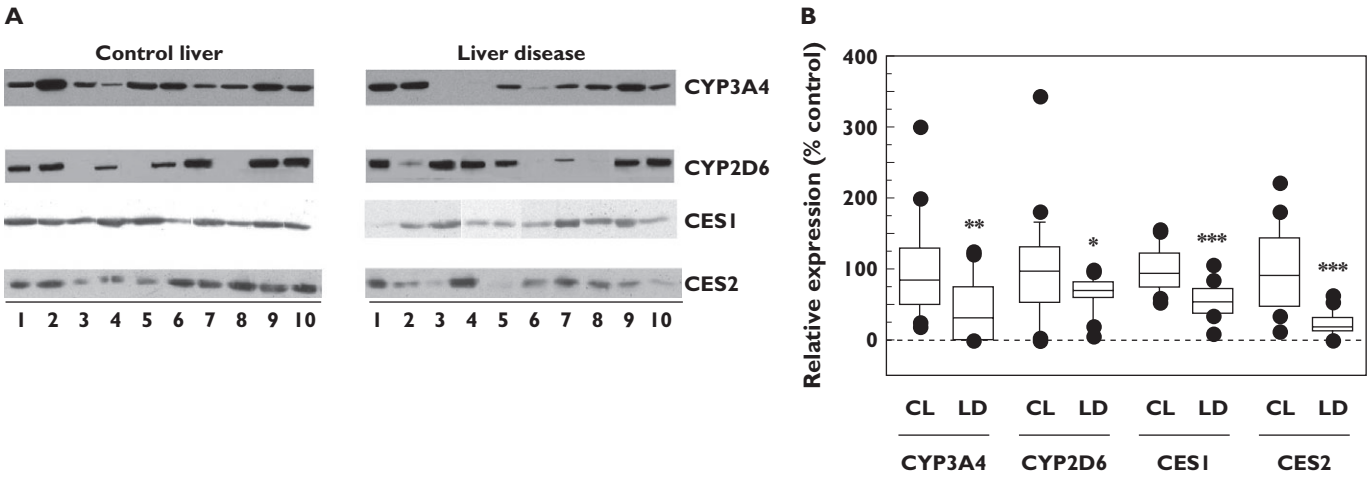

Figure 1

(A) Representative immunoblot analysis ($n = 10$ per group) of CYP3A4, CYP2D6, CES1 and CES2 expression in human hepatic microsomal fractions prepared from individuals with normal liver function (control) and liver disease. (B) Box plots showing relative expression of CYP3A4, CYP2D6, CES1 and CES2 in control liver (CL) and liver disease (LD). (The box contains the middle 50% of the data, its upper and lower edges indicate the 75th and 25th percentiles, the line within the box indicates the median and the unattached points indicate suspected outliers). Significant decrease in expression in LD relative to CL: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Mann–Whitney U-test, $n = 20$ per group)

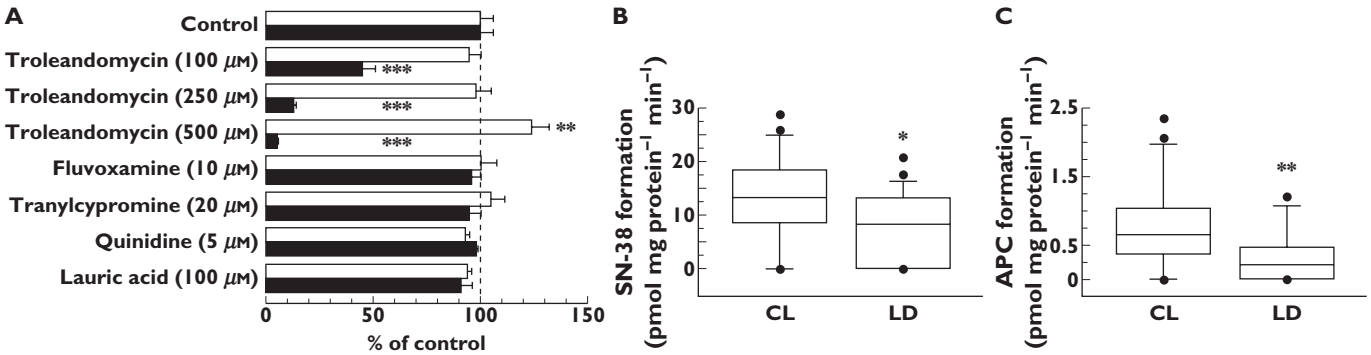

Figure 2

(A) Effects of CYP-specific inhibitors on irinotecan biotransformation in microsomal fractions from control subjects. Different from control (no inhibitor): $^{**}P < 0.01$, $^{***}P < 0.001$ (ANOVA and Protected Least Significant Difference test, $n = 3$ replicates for each treatment). The solid bars indicate APC formation and the open bars indicate SN-38 formation. Box plots showing the decrease in (B) SN-38 and (C) APC formation in hepatic microsomes from individuals with normal liver function (CL) and liver disease (LD). Significant decrease in expression in LD relative to CL: $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney U-test, $n = 20$ per group)

contrast, inhibitors of CYPs 1A2 (fluvoxamine, 10 μM), 2C (tranylcypromine, 20 μM), 2D6 (quinidine, 5 μM) and 4A11 (lauric acid, 100 μM) were inactive (Figure 2A). APC (5 μM) was not converted to SN-38 in human liver. Taken together, these findings support the selective involvement of CYP3A4 in APC formation and CES1/2 in hepatic SN-38 formation.

Irinotecan biotransformation in microsomal fractions from patients with chronic liver disease

Hepatic microsomes from 20 patients with severe liver dysfunction were available for the present study. Ten individuals were diagnosed with hepatocellular liver disease (four were Child-Pugh grade B and six were grade C) and 10 with cholestatic disease (one with Child-Pugh grade A, four with grade B and five with grade C; Table 2). The formation of SN-38 was decreased in these fractions (range 0.0 to 20.7 pmol mg protein^{-1} min^{-1}, median 8.4 pmol mg protein^{-1} min^{-1}, $P < 0.05$ compared with control Figure 2B). No apparent difference between the types of liver disease was evident (Table 2). In accord with these findings, decreases in the relative expression of CES1 (median 54% of control, range 10–105% of control; $P < 0.001$ compared with control; Figure 1B) and CES2 (median: 19% of control, range 0–64% of control, $P < 0.001$; Figure 1B) immunoreac-

Table 2

Liver disease patients: clinicopathological characteristics, drug histories and microsomal irinotecan biotransformation

	Drug history	Child-Pugh grade	Serum albumin (mg ml^{-1})	Serum bilirubin (nmol ml^{-1})	Prothrombin time (s)	SN-38 formation (pmol mg protein^{-1} min^{-1})	APC formation (pmol mg protein^{-1} min^{-1})
Hepatocellular disease							
LD1	Prednisone	C	28	72	42	13.5	0.20
LD2	Prednisone, norfloxacin, propranolol, spironolactone	B	29	18	27	0	0
LD3	Ranitidine, prednisone, insulin	C	25	488	52	0	0
LD4	Ranitidine, norfloxacin, oxazepam	C	29	23	38	15.3	0.93
LD5	Spironolactone, ciprofloxacin	C	29	52	45	12.5	0.20
LD6	Prednisone, spironolactone	C	28	32	25	0	0
LD7	Ranitidine, cefotaxime, propranolol, pethidine, hydrocortisone	C	25	389	60	8.6	0.26
LD8	Spironolactone, oxazepam, omeprazole	B	41	15	18	20.7	1.21
LD9	Sucralfate, spironolactone	B	34	89	18	0	0
LD10	Propranolol, spironolactone, frusemide, cimetidine	B	23	146	12	7.7	0.24
Median		(4B/6C)	29	62	33	8.1	0.20
Range			(23–41)	(15–488)	(12–60)	(0–20.7)	(0–1.21)
Cholestatic disease							
LD11	Norfloxacin, spironolactone	C	25	85	28	17.6	0.64
LD12	Spironolactone, rifampicin	B	34	241	18	0	0
LD13	Cefotaxime	C	24	374	18	13.4	1.21
LD14	Ciprofloxacin	B	33	96	17	8.2	0.20
LD15	Spironolactone, atenolol	C	28	211	18	9.8	0.34
LD16	Ranitidine, ampicillin, cefotaxime, frusemide, morphine	C	33	500	41	0	0
LD17	Ranitidine, propranolol	B	30	107	17	5.7	0.20
LD18	None	B	41	246	27	11.1	0.31
LD19	Spironolactone, thyroxine	C	27	52	32	13.2	0.59
LD20	Ciprofloxacin	A	40	433	18	0	0
Median		(1A,4B,5C)	32	226	18	9.0	0.26
Range			(24–41)	(52–433)	(17–41)	(0–17.6)	(0–1.21)
All liver disease							
Median		(1A,8B,11C)	29	102	26	8.4	0.20
Range			(23–41)	(15–500)	(12–60)	(0–20.7)	(0–1.21)

tive proteins were observed in liver disease samples. Similarly, APC formation was decreased in hepatic microsomes from patients with chronic liver disease (median 0.20, range 0.0 to 1.21 pmol mg protein^{-1} min^{-1}; Table 2) compared with control liver ($P < 0.01$; Figure 2C). Again both forms of liver disease affected APC formation similarly (Table 2). Immunoreactive CYP3A4 was decreased in liver disease samples (median 33% of control, range 0–126% of control, $P < 0.05$; Figure 1B) but, in contrast, CYP2D6 expression was extremely variable in both populations and the decline in liver disease was less pronounced (median 68% control, range 5–99% control; $P < 0.05$, Figure 1B). APC formation was correlated with CYP3A4 content ($\rho = 0.33$, $P = 0.05$; Figure 3A), while SN-38 formation was correlated with CES1 ($\rho = 0.38$, $P < 0.05$; Figure 3B) and CES2 ($\rho = 0.52$, $P < 0.01$; Figure 3C).

Relative SN-38 and APC formation in microsomes is related to the extent of hepatic dysfunction

The SN-38 : APC ratio was used to reflect relative CES/CYP3A activity and to assess whether the relative production of SN-38 was altered in liver disease. Wide interindividual variation in the ratio was observed between samples from patients with liver disease, with seven of 20 forming neither metabolite. In samples from three subjects that retained biotransformation capacity (LD1 and LD5, who were both Child-Pugh grade C, and LD14, who was grade B) the SN-38 : APC ratio was in the range 41–68 whereas the remaining 10 active fractions exhibited ratios in the range 11–36. The relationship between microsomal SN-38 and APC formation was highly significant ($\rho = 0.81$, $P < 0.001$; Figure 3D). From these findings it appears possible that a subset of patients who retain the capacity for phase I biotransformation of irinotecan may generate more SN-38 relative to APC, which may then lead to toxicity.

As shown in Table 2 serum albumin was decreased in donors with liver disease (median 29, range 23–41 mg ml^{-1}, compared with the normal range of 36–50 mg ml^{-1}; $P < 0.001$), while serum bilirubin was increased (median 102, range 15–500 nmol ml^{-1}, compared with the normal range of 3–15 nmol ml^{-1}; $P < 0.001$) and prothrombin time was extended (median 26, range 12–60 s, compared with the normal range of 11–17 s; $P < 0.001$). These parameters did not differ significantly in patients with either hepatocellular or cholestatic liver disease. Previous studies have suggested that irinotecan dosage should be modified in

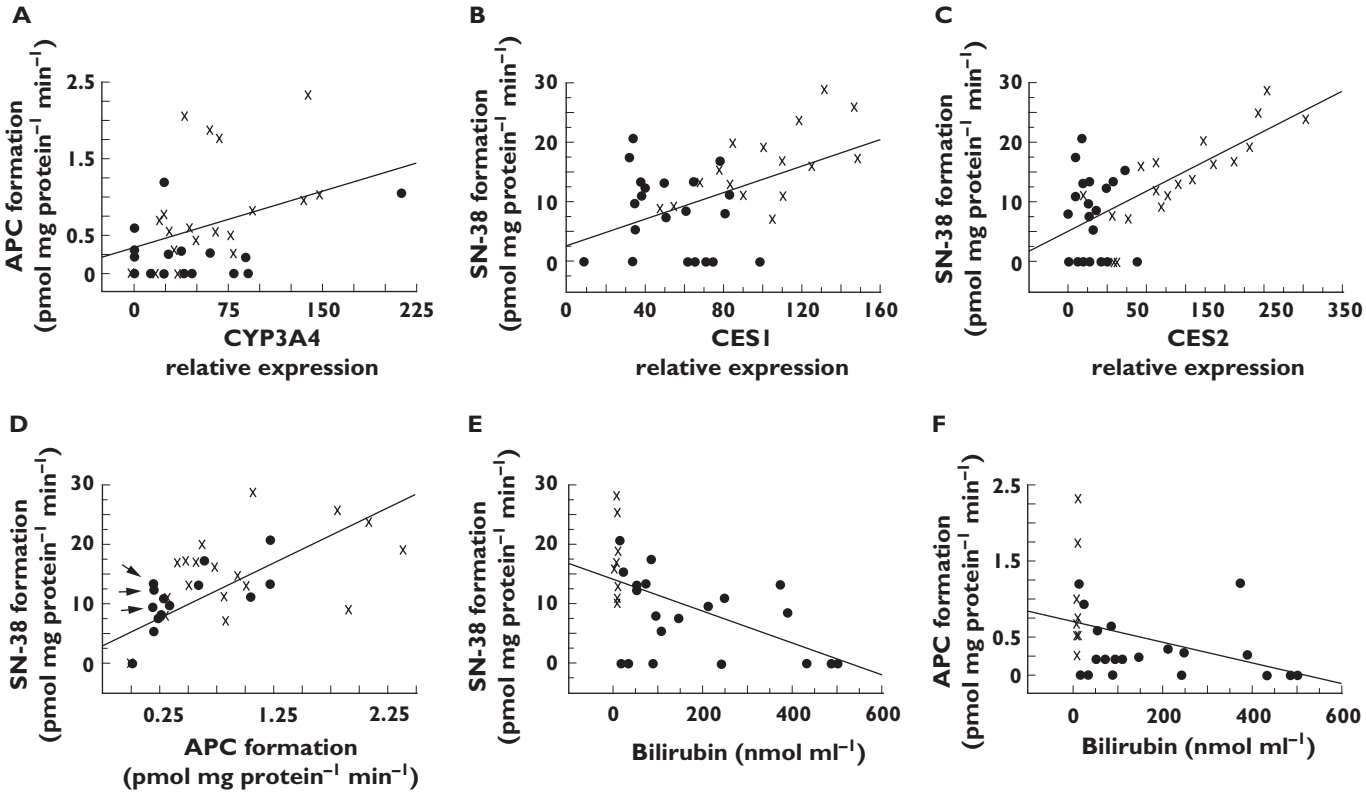

Figure 3

Relationships between (A) APC formation and relative CYP3A4 content and SN-38 formation and relative (B) CES1 and (C) CES2 content in microsomal fractions. (D) Relationship between microsomal SN-38 and APC formation; arrows indicate the liver disease samples that exhibited SN-38 : APC ratios in the range 41–68. Relationships between serum bilirubin concentration and (E) SN-38 and (F) APC formation in hepatic microsomes. In these plots individual subjects had normal liver function (X) or liver disease (●)

patients with chronic liver disease [8–13]. Therefore we assessed how the presence of disease altered the relative production of the major metabolites of irinotecan formed during the initial phase of biotransformation. As shown in Figure 3E and F, SN-38 and APC formation were inversely correlated with serum bilirubin concentrations ($\rho < 0.01$ and $\rho < 0.05$, respectively). SN-38, but not APC, formation was also inversely related to serum albumin ($P < 0.05$). Prothrombin time was not significantly correlated with the formation of either phase I metabolite of irinotecan.

Discussion

About 60% of patients with colorectal tumours develop hepatic metastases that cause liver dysfunction. Drug clearance is severely impaired in chronic liver disease because the altered hepatic lobular architecture leads to intrahepatic shunting of blood flow and because the number and residual function of hepatocytes are decreased [24, 25]. Irinotecan is a prodrug that undergoes hepatic CES-dependent activation to SN-38, as well as CYP3A4-mediated inactivation to APC. Therefore the impact of liver disease on efficacy is potentially complex. Glucuronidation is also an important deactivation pathway for SN-38 [1, 3, 5], although phase II drug conjugation is relatively preserved in liver disease providing inflammatory injury is minimal [26]. Several case reports of altered irinotecan pharmacokinetics and severe toxicity in cancer patients with liver dysfunction have appeared [8–10]. The ratio of the AUCs of SN-38 and irinotecan was correlated with serum biochemical parameters indicative of liver function [27]. Accordingly, dose-reduction is recommended if serum bilirubin concentrations exceed 1.5 times the upper limit of normal [11], which reflects the extent of liver dysfunction [13]. In the present study, impairment of microsomal irinotecan biotransformation was well correlated with serum bilirubin concentrations in patients with liver disease, but not with serum albumin or prothrombin times.

Evidence for decreased CYP3A4 function in liver disease has been presented. Clearance of the CYP3A4 substrates midazolam and erythromycin was decreased due to impaired hepatic oxidation [28, 29]. Biochemical studies have also reported a decline in CYP3A function in individuals with hepatocellular disease, although not to the same extent as for several other CYPs, such as CYP1A2 [30, 31]. *In vivo* phenotyping with the CYP3A drug substrates erythro-

mycin or midazolam revealed a close relationship with irinotecan clearance [32]. In the present study APC formation was decreased in microsomes from patients with chronic liver disease, consistent with the decline in CYP3A4 function in these fractions. By comparison, the impact of liver disease on CYP2D6 function and expression was less pronounced, possibly due to the well described polymorphism that contributes more extensively than liver disease to the inter-individual variation in biotransformation.

The present set of microsomal fractions was obtained from 'control' subjects who did not exhibit major hepatic dysfunction, and individuals with impaired liver function according to the Child-Pugh scale. This is a scoring system that weights hepatic encephalopathy and ascites, which reflect impaired toxin clearance and portal hypertension, and serum bilirubin, albumin and prothrombin times to provide gradings according to disease severity [15, 16]. Child-Pugh Grade A indicates the least severe hepatic dysfunction and has a moderate prognosis, Child-Pugh Grade B is a much more severe form of liver injury and in Child-Pugh Grade C the prognosis is extremely poor. In the present study 19 of the patients were either Child-Pugh Grades B or C, with a similar distribution between hepatocellular and cholestatic disease. Microsomal SN-38 and APC formation did not differ significantly between the two forms of cirrhosis, suggesting that disease severity may influence biotransformation to a greater extent than disease aetiology.

An increase in dose-normalized exposure to irinotecan and the active metabolite SN-38 was reported in patients with liver dysfunction [11]. Thus, it has been suggested that SN-38 formation may be increased indirectly under conditions in which APC formation is decreased, including CYP3A4 down-regulation in liver disease and CYP3A4 inhibition by selective inhibitors [33, 34]. In the present study, SN-38 formation was significantly decreased in microsomes from most patients with either hepatocellular or cholestatic cirrhosis compared with control subjects. In samples from seven of the 20 donors who had liver disease, no evidence for irinotecan biotransformation was found. This is in accord with the decline in expression of the high affinity CES2 enzyme as well as the low-affinity CES1. However, in three samples SN-38 production was higher, relative to APC formation, than in the remaining 10 active samples. This may provide additional insight into the reported increase in dose-normalized exposure to SN-38 in patients with liver dysfunction [11] and contribute to toxicity in a subset of patients. The down-regulation of CYP3A4, but relative sparing of CES1/2 and, in particular, UGT1A1 [26] in some patients with liver disease may enhance the relative formation of SN-38 and mediate toxicity.

Similarly, there have been reports that co-administration of irinotecan and the potent CYP3A4 inhibitors lopinavir, ritonavir or ketoconazole decreased APC elimination and concomitantly increased systemic SN-38 exposure up to two-fold in patients with intact liver function [29, 30]. In the present study we found a small but significant increase in SN-38 production in microsomal fractions from control subjects in the presence of high concentrations of the CYP3A inhibitor troleandomycin. These clinical and experimental observations suggest that potent CYP3A inhibition, which inhibits APC formation, may enhance the flux of substrate (irinotecan) through the CES pathway. Taken together, the preservation of CES activity, whether elicited by inhibitors or, in a subset of patients, by liver disease that preferentially decreases CYP3A expression, may enhance SN-38 formation and increase the chance of toxicity.

In summary, the present study has found that pathways of irinotecan biotransformation mediated by CYP3A and CES are both impaired in severe liver disease, which influences the rate of irinotecan bioactivation and inactivation. There are limitations to the interpretations that may be placed on findings in microsomal fractions, but the present study offers insights that are complementary to those from clinical studies. The impact of liver disease on SN-38 formation in most patients has implications for efficacy and it is feasible that extrahepatic CES activity may contribute to the formation of the active metabolite. Dose adjustments in cancer patients with liver disease who receive irinotecan should also closely monitor circulating SN-38 concentrations, especially in those receiving concurrent therapy with CYP3A4 inhibitory drugs.

Competing interests

There are no competing interests to declare.

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